

1. A soluble polypeptide which specifically binds an FKBP/rapamycin complex, which binding is rapamycin-dependent.
- 5 2. The polypeptide of claim 1, which polypeptide comprises a soluble portion of a RAPT1-like polypeptide that binds to said FKBP/rapamycin complex.
3. The polypeptide of claim 1, wherein said RAPT1-like polypeptide portion has an amino acid sequence identical or homologous with a rapamycin-binding domain represented by an amino acid sequence selected from the group consisting Val26-Tyr160 of SEQ ID
10 No. 2, Val1272-Tyr1444 of SEQ ID No. 12, Val41-Tyr173 of SEQ ID No. 14, Val1-Tyr133 of SEQ ID No. 16, and Val1-Arg133 of SEQ ID No. 18.
4. The polypeptide of claim 1, which polypeptide comprises a portion of a rapUBC-like protein that binds to said FKBP/rapamycin complex.
- 15 5. The polypeptide of claim 1, which polypeptide is a fusion polypeptide comprising a first polypeptide portion for binding to said FKBP/rapamycin complex, and a second polypeptide portion having an amino acid sequence unrelated to said first polypeptide portion.
- 20 6. The polypeptide of claim 5, wherein said second polypeptide portion provides a detectable label for detecting the presence of said fusion protein.
7. The polypeptide of claim 5, wherein said second polypeptide portion provides a matrix-binding domain for immobilizing said fusion protein on an insoluble matrix.
- 25 8. The polypeptide of claim 5, wherein said fusion polypeptide is functional in a rapamycin-dependent two-hybrid assay.
- 30 9. A soluble protein comprising a rapamycin-binding domain of a RAPT1-like polypeptide, which protein specifically binds an FKBP/rapamycin complex in a rapamycin-dependent manner.
- 35 10. The protein of claim 9, wherein said rapamycin-binding domain has an amino acid sequence identical or homologous with a rapamycin-binding domain represented by an amino acid sequence selected from the group consisting Val26-Tyr160 of SEQ ID No. 2, Val1272-Tyr1444 of SEQ ID No. 12, Val41-Tyr173 of SEQ ID No. 14, Val1-Tyr133 of SEQ ID No. 16, and Val1-Arg133 of SEQ ID No. 18.

11. A soluble polypeptide portion of a RAPT1-like protein, which polypeptide specifically binds an FKBP/rapamycin complex in a rapamycin-dependent manner.

5 12. The polypeptide of claim 11, which polypeptide is represented by the general formula X-Y-Z, wherein

Y represents a rapamycin-binding domain within residues 1272 to 1444 of SEQ ID No. 12,

10 X is absent or represents a polypeptide from 1 to about 500 amino acid residues of SEQ ID No. 12 immediately N-terminal to said rapamycin-binding domain, and

Z is absent or represents from 1 to about 365 amino acid residues of SEQ ID No. 2 immediately C-terminal to said rapamycin-binding domain.

13. A chimeric polypeptide represented by the general formula X-Y-Z, wherein

15 Y represents a rapamycin-binding domain consisting essentially of amino acid residues 1272 to 1444 of SEQ ID No. 12, or a corresponding rapamycin-binding domain of a RAPT1-like protein homologous thereto, and

X and Z are, separately, absent or represent polypeptides having amino acid sequences unrelated to a RAPT1-like protein.

20 14. A nucleic acid encoding a soluble polypeptide which specifically binds an FKBP/rapamycin complex, which binding is rapamycin-dependent.

25 15. The nucleic acid of claim 14, which polypeptide comprises a soluble portion of a RAPT1-like polypeptide that binds to said FKBP/rapamycin complex.

30 16. The nucleic acid of claim 14, wherein said RAPT1-like polypeptide portion has an amino acid sequence identical or homologous with a rapamycin-binding domain represented by an amino acid sequence selected from the group consisting Val26-Tyr160 of SEQ ID No. 2, Val1272-Tyr1444 of SEQ ID No. 12, Val41-Tyr173 of SEQ ID No. 14, Val1-Tyr133 of SEQ ID No. 16, and Val1-Arg133 of SEQ ID No. 18.

35 17. The nucleic acid of claim 14, which polypeptide comprises a portion of a rapUBC-like protein that binds to said FKBP/rapamycin complex.

18. The nucleic acid of claim 14, which nucleic acid encodes a fusion polypeptide comprising a first polypeptide portion for binding to said FKBP/rapamycin complex,

and a second polypeptide portion having an amino acid sequence unrelated to said first polypeptide portion.

5 19. The nucleic acid of claim 18, wherein said second polypeptide portion provides a detectable label for detecting the presence of said fusion protein.

20. The nucleic acid of claim 18, wherein said second polypeptide portion provides a matrix-binding domain for immobilizing said fusion protein on an insoluble matrix.

10 21. The nucleic acid of claim 18, wherein said fusion polypeptide is functional in a rapamycin-dependent two-hybrid assay.

15 22. A soluble polypeptide portion of a RAPT1-like protein, which polypeptide specifically binds an FKBP/rapamycin complex in a rapamycin-dependent manner, and is represented by the general formula X-Y-Z, wherein

Y represents a rapamycin-binding domain of a yeast RAPT1-like protein,

X is absent or represents a polypeptide from 1 to about 200 amino acid residues of the RAPT1-like protein immediately N-terminal to said rapamycin-binding domain, and

20 Z is absent or represents from 1 to about 200 amino acid residues of the RAPT1-like protein immediately C-terminal to said rapamycin-binding domain.

23. The polypeptide of claim 22, wherein the rapamycin-binding domain comprises a polypeptide represented by an amino acid sequence selected from the group consisting of Val41-Tyr173 of SEQ ID No. 14, Val1-Tyr133 of SEQ ID No. 16, and Val1-Arg133 of SEQ ID No. 18.

24. A chimeric polypeptide represented by the general formula X-Y-Z, wherein
30 Y represents a rapamycin-binding domain consisting essentially of amino acid residues Val41-Tyr173 of SEQ ID No. 14, Val1-Tyr133 of SEQ ID No. 16, or Val1-Arg133 of SEQ ID No. 18, or a corresponding rapamycin-binding domain of a yeast or fungal RAPT1-like protein homologous thereto, and
X and Z are, separately, absent or represent polypeptides having amino acid sequences unrelated to a RAPT1-like protein.

35 25. A substantially pure preparation of an RAPT1 polypeptide, or a fragment thereof, having an amino acid sequence at least 70% homologous to SEQ ID NO. 2 or 12.

26. The polypeptide of claim 25, wherein said polypeptide binds to an FKBP/rapamycin complex.
27. The polypeptide of claim 25, having an amino acid sequence at least 95% homologous to the amino acid sequence of SEQ ID No. 2 or 12.
28. The polypeptide of claim 25, wherein said polypeptide functions in one of either role of an agonist of rapamycin regulation of cell proliferation or an antagonist of rapamycin regulation of cell proliferation.
29. The polypeptide of claim 25, wherein said polypeptide comprises a phosphatidylinositol kinase activity.
30. The polypeptide of claim 25, wherein said polypeptide is a recombinant protein produced from a pIC524 clone of ATCC deposit 75787.
31. The polypeptide of claim 25, wherein polypeptide is of mammalian origin.
32. An immunogen comprising the polypeptide of claim 25, in an immunogenic preparation, said immunogen being capable of eliciting an immune response specific for said RAPT1 polypeptide.
33. An antibody preparation specifically reactive with an epitope of the immunogen of claim 32.
34. A recombinant RAPT1 polypeptide, or a fragment thereof, having an amino acid sequence at least 70% homologous to SEQ ID NO. 2 or 12.
35. The polypeptide of claim 34, wherein said polypeptide binds to an FKBP/rapamycin complex.
36. The polypeptide of claim 34, wherein said polypeptide functions in one of either role of an agonist of rapamycin regulation of cell proliferation or an antagonist of rapamycin regulation of cell proliferation.
37. The polypeptide of claim 34, wherein said polypeptide is expressed from recombinant gene produced from a pIC524 clone of ATCC deposit 75787.

38. The polypeptide of claim 34, which polypeptide is a fusion protein further comprising, a second polypeptide portion having an amino acid sequence from a protein unrelated to the protein of SEQ ID No. 2 or 12.

5 39. The polypeptide of claim 38, wherein said fusion protein is functional in a two-hybrid assay.

10 40. A substantially pure nucleic acid having a nucleotide sequence which encodes RAPT1 protein, or a fragment thereof, having an amino acid sequence at least 70% homologous to SEQ ID NO. 2 or 12.

15 41. The nucleic acid of claim 40, further comprising a transcriptional regulatory sequence operably linked to said nucleotide sequence so as to render said nucleotide sequence suitable for use as an expression vector.

42. An expression vector, capable of replicating in at least one of a prokaryotic cell and eukaryotic cell, comprising the nucleic acid of claim 40.

20 43. A host cell transfected with the expression vector of claim 42 and expressing said polypeptide.

25 44. A method of producing a recombinant RAPT1 protein comprising culturing the cell of claim 43 in a cell culture medium to express said RAPT1 protein and isolating said RAPT1 protein from said cell culture.

45. The recombinant gene of claim 40, wherein said recombinant gene is functional in a two-hybrid assay.

30 46. An assay for screening test compounds for agents which induce the binding of a RAP-binding protein with an FK506-binding protein, comprising

i. combining

a RAP-BP polypeptide comprising a rapamycin-binding domain represented by an amino acid sequence SEQ ID No. 2 or 12, and

a FKBP polypeptide comprising a rapamycin-binding domain of an FK506-binding protein

35 under conditions wherein said RAP-BP and FKBP polypeptides are able to interact;

ii. contacting said combination with a test compound; and

- iii. detecting the formation of a complex comprising said RAP-BP and FKBP polypeptides,

wherein an increase in the formation of said complex in the presence of said test compound is indicative of an inducer of the interaction between a RAP-binding protein with an FK506-binding protein.

47. A method for screening test compounds for agents which induce the binding of a RAP-binding protein with an FK506-binding protein, comprising

(i) providing a host cell containing a detectable gene wherein the detectable gene expresses a detectable protein when the detectable gene is activated by an amino acid sequence including a transcriptional activation domain when the transcriptional activation domain is in sufficient proximity to the detectable gene;

(ii) transforming the host cell with a first chimeric gene that is capable of being expressed in the host cell, the first chimeric gene comprising a DNA sequence that encodes a first hybrid protein, the first hybrid protein comprising:

(a) a DNA-binding domain that recognizes a binding site on the detectable gene in the host cell; and

(b) a rapamycin-binding domain of an FK506-binding protein;

(iii) transforming the host cell with a second chimeric gene that is capable of being expressed in the host cell, the second chimeric gene comprising a DNA sequence that encodes a second hybrid protein, the second hybrid protein comprising:

(a) the transcriptional activation domain; and

(b) a rapamycin-binding domain of a RAPT1-like protein;

(iv) subjecting the host cell to conditions under which the first hybrid protein and the second hybrid protein are expressed in sufficient quantity for the detectable gene to be activated;

(v) contacting the host cell with a test agent; and

(vi) determining whether the detectable gene has been expressed to a degree statistically significantly greater than expression in the absence of an interaction between the first test protein and the second test protein.

48. The method of claim 47, wherein the DNA-binding domain and transcriptional activation domain are derived from transcriptional activators having separable DNA-binding and transcriptional activation domains.

49. The method of claim 48, wherein the DNA binding domain and the transcriptional activation domain are selected from the group consisting of transcriptional activators GAL4, GCN4, LexA, VP16 and ADR1.

5 50. The method of claim 47, wherein the rapamycin-binding domain of the FK506-binding protein is part of the second hybrid protein rather than the first hybrid protein and the rapamycin-binding domain of the RAPT1-like protein is part of the first hybrid protein rather than the second hybrid protein.

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